CULTURE MEDIA FOR GONOCOCCUS *

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HISTORICAL REVIEW

In 1879 Neisser[1] discovered Gonococcus. Bumm[2] (1885) was the first of many investigators to obtain satisfactory results in its cultivation. He used human placental blood serum as the medium. Wertheim[3] (1891) obtained a luxuriant growth using serum agar. As substitutes for human serum, several protein-containing fluids and animal serum have been tried by many workers; for example, hydrocele fluid by Steinshneider[4] (1890), ovarian, cystic, and hydrosalpinx fluid by Menge[5] (1893), and ascites and hydrothorax fluid by Kiefer[6] (1895). Scholtz[7] noticed that the amount of protein in these fluids varied, so that the gonococcal growth was not constant, while Laitiner reported that the alkalinity of cystic and ascitic fluid has a great influence on the growth. J. Koch[8] recorded fair results with ascitic agar, and Wassermann[9] with pig serum nutrose agar. Stross,[9] in a comparative study of human and animal sera, found that the gonococcus grew readily on human serum but not on animal serum. Schrötler and Winkler[10] (1890) used peewit protein with satisfactory results. Preparations of proteins of other kinds were used by Finger, Ghon, and Schlagenhaufer,[11] protein-containing urine agar by De Christmas,[12] egg yoke by Steinschneider,[13] and egg-white agar by Lipschütz,[14] but none of these media was satisfactory in the hands of other workers. Abel[15] introduced Peiffer's blood agar, and later J. Koch used horse-blood agar with excellent results. M. Seé[16] found rabbit-blood agar unsatisfactory. Thalman[17] was the first to secure growth of the gonococcus on ordinary culture medium; he pointed out that plain agar must be exactly neutralized. Later his work was corroborated by Brongersma and Van de Velde,[18] Picker,[19] and others, but Baermann,[20] Rotmann,[21]

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1 Centralbl. f. d. med. Wissensch., 1879, 17, p. 497.
2 Deutsch. med. Wchnschr., 1885, 11, p. 508.
3 Ibid., 1891, 17, p. 1351.
4 Berlin klin. Wchnschr., 1897, 34, p. 379.
5 Zentralbl. f. Gynäk., 1893, 17, p. 153.
6 Berlin klin. Wchnschr., 1895, 32, p. 332.
7 Arch. f. Dermat. u. Syph., 1899, 49, p. 3.
8 Kolle and Wassermann's Handb. d. pathogen. Mikroorganismen, 1912, 4, p. 655.
9 Berlin klin. Wchnschr., 1897, 34, p. 685. Ztschr. f. Hyg. u. Infektionskrankh., 1898, 27, p. 298.
10 Centralbl. f. Bakteriol., I, O., 1905, 38, p. 491.
11 Arch. f. Dermat. u. Syph., 1894, 28, p. 3.
12 Ann. de l'Inst. Pasteur, 1897, 9, p. 609; 1900, 14, p. 331.
13 Berlin klin. Wchnschr., 1895, 32, p. 984.
14 Centralbl. f. Bakteriol., I, O., 1904, 36, p. 743.
15 Deutsch. med. Wchnschr., 1893, 19, p. 265.
16 Ann. de dermat. et de syph., 1900, 31, p. 889.
17 Centralbl. f. Bakteriol., I, O., 1900, 27, p. 828; 1902, 31, p. 678.
18 Centralbl. f. Bakteriol., I, O., 1903, 33, p. 311.
19 Wien. klin. Wchnschr., 1906, 19, p. 122.
20 Ztsch. f. Hyg. u. Infektionskrankh., 1904, 43, p. 529.
21 Monatsh. f. prakt. Dermat., 1905, 41, p. 516.

Alfvén,[22] and Jackstadt[23] did not obtain the same results. Urbahn,[24] Wildbolz,[25] Vannod,[26] Martin,[27] and others, succeeded in getting a growth on ordinary agar. Of these, Vannod noted that slight alkalinity of the medium was necessary. Recently Alcock reported a luxuriant growth of the gonococcus on ordinary peptone agar. In 1913 Sabouraoud and Noire,[28] and later Weil and Noire,[29] used whey agar successfully. In the same year Besredka and Jupille[30] found egg broth a suitable medium.

According to the investigations cited, we have no really satisfactory media for the cultivation of the gonococcus. I have hitherto used horse- or goat-blood agar with satisfactory results. Recently, however, I have prepared and used, as a substitute for blood agar, the medium to be described.

THE PREPARATION OF THE MEDIUM

Two hundred cubic centimeters of cow's milk are warmed to 60 C. and 5% ascitic agar is added drop by drop, the milk being shaken to cause precipitation of the casein. It is then filtered through filter paper. To the filtrate is added 10% caustic-soda solution up to the point of slight alkalinity, and then 2 gm. of urea. The clear colorless fluid is sterilized at 60 C. for 30 minutes every day for 3 days. The sterilized fluid is then mixed with melted 0.3% nutrose agar at 45 C. in the proportion of 1 part of the fluid to 2 parts of agar. Plate or slant forms are prepared as needed. The medium must be incubated before use to make sure that no micro-organisms have gained access during preparation. When fluid media are needed, equal parts of the fluid and of ordinary broth or peptone water are mixed.

It is very necessary that the fluid should not be heated above 70 C., as unsatisfactory results frequently follow the use of the fluid heated at a higher temperature. For the precipitation of casein the amount of acid is not constant, the different samples of milk undoubtedly varying in quality.

APPEARANCE OF THE GONOCOCCUS IN CULTURE

Six strains were cultivated, 4 of which grew well after 48 hours at 37 C. and 2 after 5 days. The results with the four strains may be outlined as follows:

1. Whey agar at 37 C. After 24 hours the gonococcus colony was macroscopically hardly visible, round, about 1 mm. in diameter—a slightly elevated disc with moist-looking surface, semitransparent, of smooth margin, and of light grayish-blue color. After 48 hours, the colony, macroscopically, was small, round, about 2 mm. in diameter—

[22] Hygiea, 1904, 66, p. 151.
[23] Dissertation, Königsberg, 1904.
[24] München. med. Wchnschr., 1903, 50, p. 1529.
[25] Centralbl. f. Bakteriol., I, O., 1902, 31, p. 128.
[26] Ibid., 1905, 44, pp. 10, 110.
[27] Jour. Path. and Bacteriol., 1910, 15, p. 76.
[28] Ann. de dermat. et de syph., 1913, 4, p. 439.
[29] Compt. rend. de Soc. de biol., 1913, 74, p. 1321.
[30] Ann. de l'Inst. Pasteur, 1913, 27, p. 1009.

an elevated disc of viscous consistency. When touched with a platinum loop the colonies were readily removed from the medium. Surface moist and shining. Semitransparent. Margin smooth. Color grayish-blue. Centers of colonies light-brown. Microscopically the colony was a finely granular, elevated disc with uniform margin, and of a faint yellowish-dark color. Center of colony humpy and opaque. After 72 hours, macroscopically, the colony was still small and round —an elevated disc of sticky consistency, harder to remove from the medium. Margin somewhat dried and undulated. Color gray. Center opaque. Microscopically it was granular and a little coarser, with iridescent surface and wavy margin. Yellow-dark in color. Center opaque. After 96 hours, 120 hours, 5 days, and 6 days the appearance of the colony was unchanged except that after 120 hours the surface was considerably enlarged and flat.

2. Whey broth. After from 24 to 48 hours the fluid of all strains was clear and colorless, but finely granular grayish-white sediments appeared. After from 4 to 6 days the fluid was still clear and the colonies appeared as fine crummy particles, which readily sank to the bottom when lightly shaken.

3. Whey peptone water. In this medium the growth was not so good as that in the whey broth just described.

4. Whey sugar litmus medium. A growth similar to that in whey peptone water.

CONCLUSIONS

Blood agar and blood broth are still the most suitable culture media for growth of the gonococcus. If blood cannot easily be obtained, however, whey agar or whey broth can be used as substitutes for blood agar and blood broth.